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Separation of "Uncharged" Oligodeoxynucleotide Analogs by Anion-Exchange Chromatography at High pH

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Ion-exchange chromatography is a well-established method for the analysis and purification of phosphodiester-linked oligonucleotides (1). If elution is carried out under alkaline conditions, the secondary structure of G- and C-rich oligomers is disrupted. Furthermore, elution times become more sensitive to the G and T content of the oligomer, because G and T are deprotonated at pH 10 (2). In recent work on peptide-nucleic acids (PNAs)¹ we noted that mixtures of PNA oligomers G₄, G₆, G₈, and G₁₀ are readily separated by elution at pH 12 on an RPC-5 column (3). Here we show that this separation method is more generally applicable.

The sequences of the oligomers of the type PNA-Lys-

¹ Abbreviation used: PNAs, peptide-nucleic acids.

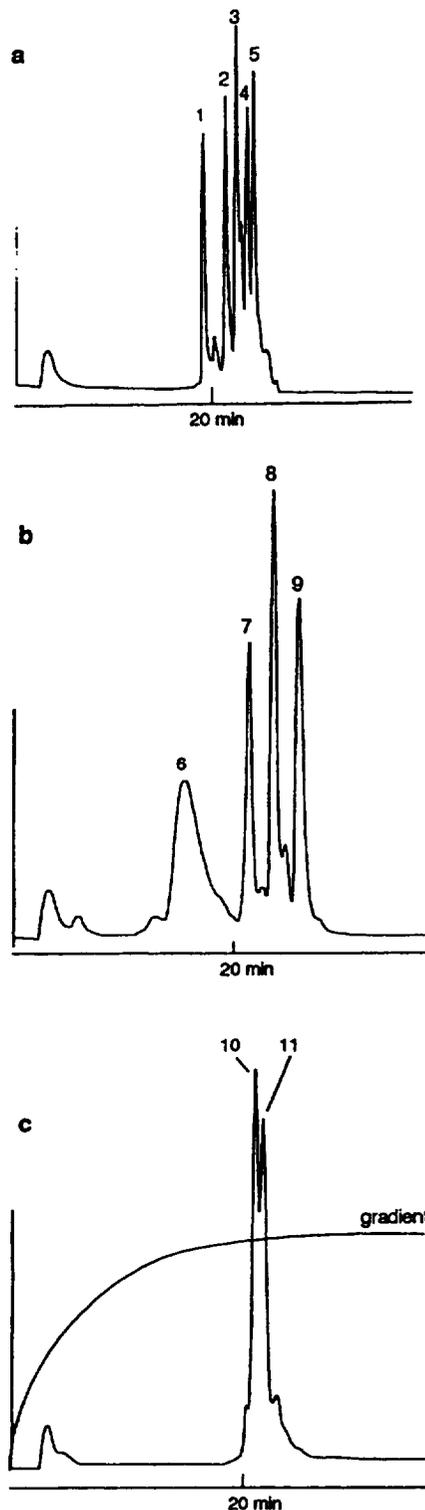


FIG. 1. Elution profiles of (a) Mixture I (samples 1-5) separated using a linear gradient from A to A/B:80/20 in 40 min; (b) Mixture II (samples 6-9) separated using a linear gradient from A to A/B:90/10 in 50 min; and (c) Mixture III (samples 10 and 11) separated on a degressive gradient, as shown in the figure, from A to A/B:96/4 in 40 min.

TABLE I
List of Sequences, T/G Contents, and Overall Charges of the PNAs Used in This Study

Mixture No.	Fig. No.	PNA No.	Sequence	T	G	Length/"charges"
I	1	1	T ₄ C ₂ TCTC	6		10/6
I	1	2	T ₂ CT ₂ CT ₄	8		10/8
I	1	3	T ₄ CT ₅	9		10/9
I	1	4	T ₁₀	10		10/10
I	1	5	T ₄ G ₂ TGTG	6	4	10/10
II	2	6	GAGAGGA ₄		4	10/4
II	2	7	A ₄ G ₂ TGAG	1	4	10/5
II	2	8	A ₃ TG ₂ TGAG	2	4	10/6
II	2	9	TGTACGTCACAACTA	4	2	15/6
III	3	10	GTAGATCACT	3	2	10/5
III	3	11	AGTGATCTAC	3	2	10/5

Note. Each oligomer has a lysine amide group attached to its carboxyl terminus.

NH₂ (4–6) used in this work are listed in Table 1. Analytical anion-exchange chromatography was carried out on an RPC-5 column as previously described (2, 7, 8), using 20 mM NaOH and 1 mM Tris-HClO₄ in water as the A buffer and 20 mM NaOH, 1 mM Tris-HClO₄, and 0.1 M NaClO₄ as the B buffer. Stock solutions of PNA oligomers containing about 5×10^{-3} ODU/ μ l at 254 nm were prepared. Sample mixtures for analysis contained 1 μ l of each relevant stock solution in 1 ml total volume. The gradients used are described in the figure legend. Peaks were assigned on the basis of their retention times and the assignments were confirmed by co-injection.

The components of mixture I separated principally on the basis of charge (the number of G and T residues) (Fig. 1a). However, the replacement of T by G resulted in an increased retention time (Fig. 1a, peaks 4 and 5). The elution profile for mixture II, which contains G-rich oligomers, shows that the separation method is probably restricted to oligomers containing 4 or more ionizable residues (Fig. 1b) since the peak corresponding to PNA 6 has a short retention time and is very broad. A pair of oligomers with the same base composition but different sequences was resolved successfully (Fig. 1c).

HPLC at alkaline pH is not a useful technique for the purification of unmodified PNAs. PNA rearranges slowly at neutral pH and more rapidly under alkaline conditions via the attack of the main-chain terminal amino group on the carbonyl function of the adjacent side chain (9). We were able to resolve PNA G₆ from its rearrangement product and hence to determine that the half-life for the rearrangement at room temperature is somewhat less than 2 h at pH 12 and about 5 days at pH 10. With longer oligomers, PNA and its rearrangement product are not resolved. Modified PNA suitable for practical applications could be purified by HPLC in the pH range 10–12.

PNAs are generally quite water soluble, but reduced

solubility is observed with longer (15–20 bases), purine-rich PNAs (10), and some PNAs also show a tendency to aggregate. Other uncharged oligonucleotide analogs are often sparingly soluble in neutral aqueous solution and tend to adopt very stable self-structures. This makes chromatography of PNA and other uncharged oligomers less effective than it is for standard DNA oligomers. Elution at a pH of 10 or greater helps to overcome these difficulties. The method should be applicable to any DNA analog that is sufficiently stable at pH 10.

The RPC-5 column that we use routinely could be replaced by a commercially available, alkali-stable anion-exchange column such as the Mono-Q column from Pharmacia (11), enabling separations on analytical or preparative scales. Finally, we believe that cation-exchange chromatography under acidic conditions could probably be used in an analogous way, since C and A residues are protonated in the pH range 3–4 and many oligonucleotide analogs including PNA are stable against depurination under acidic conditions.

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